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ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
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TABLE OF CONTENTS

LIST OF FIGURES

TABLE

FOREWORD
(Nontechnical summary)

One of the causes of death in mammals exposed to ionizing radiation is a failure of the body to produce a sufficient amount of new blood cells. Previous experiments have shown that acute stimulation of the blood-forming elements either just before or just after radiation exposure can result in less damage to, and/or more rapid recovery of, those elements. The present study examines the effect of a prolonged stimulation to produce new blood cells on the survival of lethally irradiated animals. The results indicate that at radiation exposures between 675 R and 775 R mice stimulated by 3-4 weeks exposure to reduced oxygen levels prior to irradiation survive better than unstimulated controls. Conversely, if the stimulation occurs after irradiation, survival is poorer as shown by our studies.

ABSTRACT

Experiments have been carried out to evaluate the effect of erythropoietic stimulation on postirradiation survival. A/He and Swiss mice were stimulated to produce red cells at a greater than normal rate through the use of a high altitude chamber. The response to stimulation both before irradiation and after irradiation was examined and compared with that shown by simultaneously irradiated but unstimulated control animals. To avoid the possibility that the effects to be seen might be related to tissue hypoxia during, before, or immediately after the time of irradiation, the animals were irradiated under normal atmospheric conditions. The mice also were maintained at a normal atmosphere for about 3 hours after irradiation in the case of the postirradiation stimulation and for 3 days prior to irradiation in the case of the preirradiation stimulation. The effect of postirradiation stimulation was tested at 700 and 775 R. The poststimulated mice demonstrated poorer survival than the unstimulated controls. These doses were 100 percent lethal at 30 days to both groups, however the mean survival time of the irradiated, stimulated mice was shorter than that of irradiated, unstimulated controls, 4.9 days versus 5.9 days respectively at 775 R, and 5.9 days versus 7.4 days at 700 R. In contrast, mice which were previously erythropoietically stimulated by 3 weeks continuous exposure to one-half atmosphere exhibited a markedly better postirradiation survival than did the unstimulated controls. The effect of preirradiation stimulation was tested at 675 R, 725 R and 775 R. Increased 30-day survival of the prestimulated animals over that of the untreated controls was found at all doses. The 30-day survival

percentages were as follows: at 675 R, 68 percent of the stimulated versus 24 percent of the controls; at 725 R, 36 percent of the stimulated versus 4 percent of the controls; at 775 R, 16 percent of the stimulated versus 0 percent of the controls.

I. INTRODUCTION

Previous studies have shown that one of the more critical tissues involved in lethality following radiation exposure is the blood-forming, or hematopoietic, tissue.[4] The importance of the hematopoietic tissue in radiation lethality is that the minimum radiation dose required to produce lethal damage in blood-forming tissue is lower than that required to produce lethality from any other cause. In addition, the levels of radiation exposure which can and do cause death from hematopoietic failure often have little clearly demonstrable effect on the other systems. Therefore, in order to insure recovery of a lethally irradiated man or animal, no matter what the dose, it is necessary that one insure recovery of the hematopoietic system.

Experiments with animals have shown that recovery from irradiation in the hematopoietic lethality range can be induced through the transplant of hematopoietic tissue.[2,19] The use of chemoprotective agents, particularly sulfhydryl containing compounds,[3] has also been proven successful in eliciting recovery. On the other hand, both of these techniques have certain innate difficulties. Tissue transplant in animals,[4] and possibly also in man,[11] may result in a lethal immune reaction of the transplanted cells against the host. The use of chemical protectants has the drawback that they must be administered in near-lethal amounts and within a narrow, critical period of time prior to irradiation.[3]

On the tissue level, Jacobson et al.,[9] have demonstrated that the erythroid system of rabbits stimulated by bleeding or phenylhydrazine administration prior to irradiation shows less sensitivity to irradiation and quicker recovery from a whole-body exposure of 800 R x ray than that seen in untreated, but irradiated control animals.

Stohlman et al.,[17] have obtained similar results in rats and dogs following acute bleeding within 24 hours either before or after irradiation, although acute blood loss after this time had no appreciable beneficial effect. Schack and MacDuffee[15] have also demonstrated this type of behavior in the erythroid bone marrow of mice. These investigators subjected mice to partial anoxia before x-ray exposure, until the animals showed an 80 percent increase in marrow erythroid cells. Subsequent to irradiation (500 R) the mice were maintained under normal atmospheric conditions. Their results showed a significantly more rapid recovery of erythroid cells in the irradiated mice which had been previously subjected to anoxia than in untreated, irradiated controls.

On the level of whole animals, it has been previously reported by Newsom et al.,[14] that rats subjected to reduced pO_2 following irradiation show an increased radiation sensitivity. Other work in this area indicates that these animals are under a stress to produce new red cells in order to compensate for the hypoxia to which they are exposed.[1,16] Conversely, animals stimulated to produce new red cells by acute bleeding within 24 hours before or after irradiation have been shown to exhibit a lessened sensitivity.[8]

In the studies reported here an attempt has been made to examine the problem of changing hematopoietic sensitivity and recovery through chronic alteration in the metabolic function of the hematopoietic tissue due to treatment either before or after irradiation. To accomplish this we have respectively increased or decreased the requirement for the production of new red blood cells, independent of the initial

blood cell levels and of any effects of radiation itself. This has been accomplished through an alteration in the pO_2 to which the animals are exposed prior to irradiation or following irradiation.

II. PROCEDURE

Preirradiation Stimulation

Male Swiss mice were placed in a high altitude chamber for 3 to 4 weeks. The pressure in the chamber was maintained at about one-half atmosphere. Three days before irradiation the mice were removed from the chamber and returned to normal atmospheric pressure. The mean hematocrit of these animals at the time of irradiation was 74.6 ± 3.4. Normal animals from the same age group as the altitude-subjected mice were irradiated as controls simultaneously with the altitude-treated animals. The mean hematocrit of the control animals at the time of irradiation was 54.5 ± 4.1. At the time of irradiation the age of the mice was 8 to 10 weeks. In order to prevent early deaths due to Pseudomonas infection, all animals were injected subcutaneously daily with 2 mg gentamicin sulfate (Schering) and, at separate sites, with 5 mg streptomycin sulfate according to the method of Wolf et al.[21] Antibiotic injection was begun 3 days before irradiation and terminated on the 13th day after irradiation. X-ray exposure was accomplished on a 30 mA, 250 kVp x-ray generator at an exposure rate of 20 R/min (midline air dose) and with a 0.95 mm Cu + 1.2 mm Be filter (HVL = 1.92 mm Cu). Exposure was bilateral, with the exposure rack being rotated at the midpoint of the run. Twenty-five stimulated and 25 control mice were exposed at each radiation exposure level. Following irradiation the mice were housed in pairs in shoebox-type cages. Both before and after irradiation the mice were maintained ad

libitum on standard mouse diet and on sterilized tap water to which had been added 6 ml/liter of 1N hydrochloric acid.

Postirradiation Stimulation

Eight- to 10-week old male A/He mice were subjected to one-half atmosphere of pressure for 3 days, at which time, according to the data of Gurney et al.,[7] it could be assumed that their erythropoietic activity would be approaching a significantly elevated level, but before there could occur any marked change in the peripheral red cell number. Approximately 2 hours before irradiation, the mice were removed from the chamber in order to accustomize them to normal atmospheric conditions. The animals were irradiated under normal atmospheric conditions and approximately 3 hours after irradiation were returned to one-half atmosphere pressure. Space limitations in the altitude chamber precluded individual caging or pairing of the animals. Hence, both the animals subjected to reduced pO_2 levels and their controls, which were maintained at normal atmospheric conditions, were kept six to a cage. A total of 12 stimulated and 12 control animals was utilized at each radiation exposure level. To insure a maximum level of chronic stimulation, the chamber was not opened until the 3rd day after irradiation. This precluded daily antibiotic administration. Thereafter, the chamber was opened daily for about 1 hour to allow for removal of dead animals.

Irradiation, food and water procedures were identical to those described above.

III. RESULTS

Figures 1, 2 and 3 show the survival patterns for the prestimulated (polycythemic) mice and their controls. Survival following exposure to 675, 725 and 775 R

respectively is represented. In all three cases, survival was enhanced by 3 to 4 weeks erythropoietic stimulation of the mice before exposure. Survival at 30 days following a dose of 675 R was 68 percent for the prestimulated (polycythemic) mice

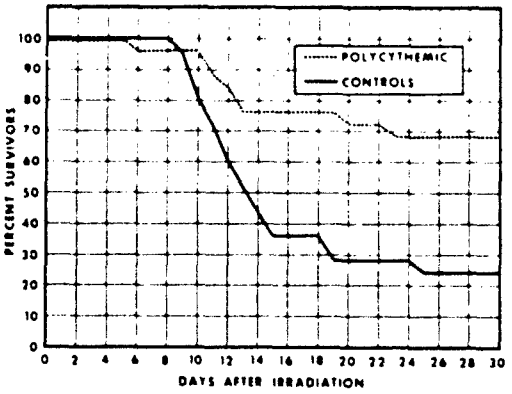

Figure 1. Effect of preirradiation erythropoietic stimulation on survival following 675 R whole-body x irradiation. (Stimulated animals are polycythemic at time of irradiation.)

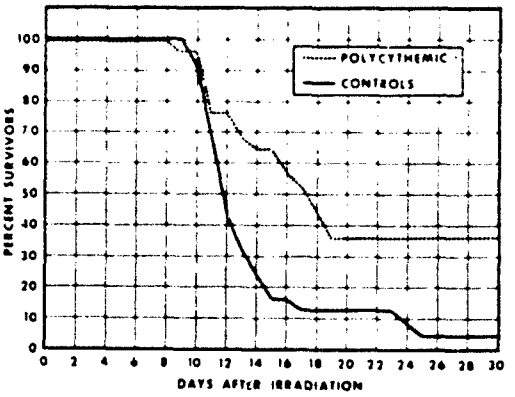

Figure 2. Effect of preirradiation erythropoietic stimulation on survival following 725 R whole-body x irradiation. (Stimulated animals are polycythemic at time of irradiation.)

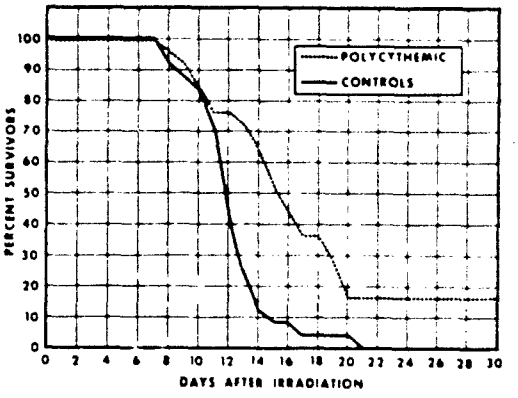

Figure 3. Effect of preirradiation erythropoietic stimulation on survival following 775 R whole-body x irradiation. (Stimulated animals are polycythemic at time of irradiation.)

versus 24 percent for the controls. A similar comparison at 725 R yields the figures of 36 percent versus 4 percent, and at 775 R, 16 percent versus 0 percent. Relative median survival times (i.e., the time at which exactly 50 percent of the animals are still alive) are given in Table I.

Table I. Median Survival Times

Treatment	Dose (R)	Treated (days)	Control (days)
Prestimulation	675	> 90	13.25
	725	17.13	11.75
	775	15.25	11.75
Poststimulation	700	4.33	6.50
	775	3.67	5.50
Antibiotic	775	11.75	9.50

Figure 4 illustrates the effect of gentamicin-streptomycin treatment versus sham saline injection. The results confirm the findings of Wolf et al.,[21] with respect to the prevention of early deaths by gentamicin-streptomycin treatment following irradiation of normal animals, when the mice are kept at no greater concentration than two per cage. At 775 R the earliest death in the antibiotic-treated animals is seen to occur at 8 days versus the significant number of 5-day deaths seen in the sham, saline-injected controls. Median survival times (Table I) are also correspondingly altered.

Figures 5 and 6 illustrate the survival patterns of poststimulated (stressed) mice. At 700 R survival has been shortened in the stressed animals throughout the entire course of the experiment. At 775 R the same general situation exists, although the differences between stressed and controls is not as great and there

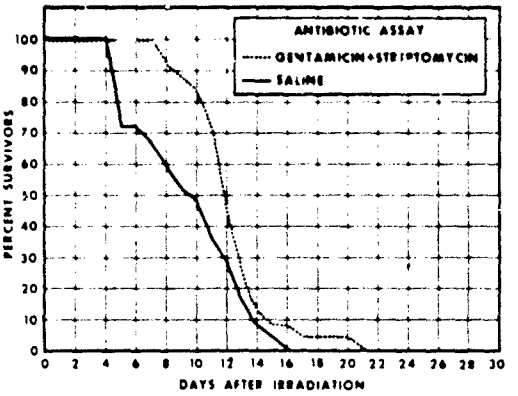

Figure 4. Effect of antibiotic injection on survival following 775 R whole-body x irradiation

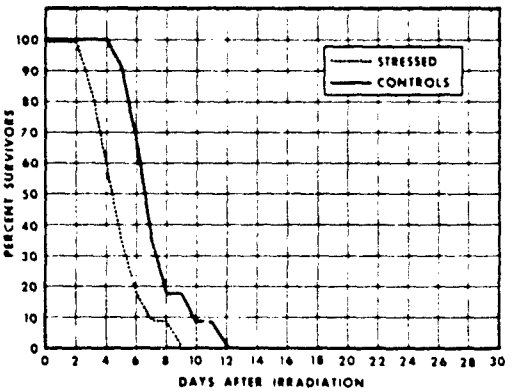

Figure 5. Effect of postirradiation erythropoietic stimulation on survival following 700 R whole-body x irradiation. (Stimulated animals are under chronic stress to produce new red cells following irradiation.)

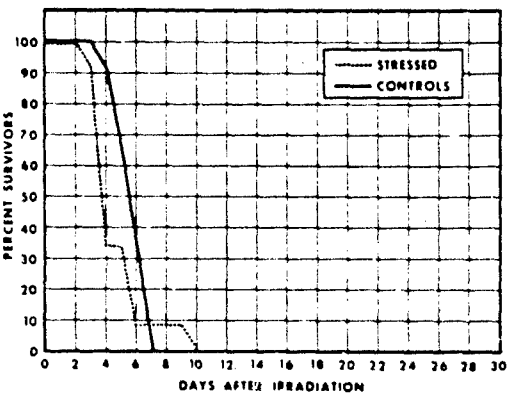

Figure 6. Effect of postirradiation erythropoietic stimulation on survival following 775 R whole-body x irradiation. (Stimulated animals are under chronic stress to produce new red cells following irradiation.)

is in fact a cross-over at 7 days following exposure. It is important to also note that, as contrasted with the prestimulated mice, there were no survivors whatsoever in either the controls or the stressed animals of the poststimulated group. In this

latter group, the maximum survival time was 12 days at 700 R and 10 days at 775 R. The median survival times were also decreased at both exposure levels (Table I).

IV. DISCUSSION

Examination of the present data indicates that preirradiation chronic erythropoietic stimulation significantly lowers sensitivity, while postirradiation chronic stimulation enhances it. The latter observation is in agreement with the earlier findings of Newsom et al.,[14] in rats exposed to hypoxia after irradiation. It should also be noted, however, that the median survival time associated with the poststimulated group and their controls as observed in this experiment is not that usually associated with the bone marrow syndrome.[4] Rather, it is more characteristic of a gut syndrome or a bacteremia syndrome. Throughout the experiment, heart blood culture tests were made on animals whenever possible within an hour of their time of death. However, since deaths occurred randomly, assay of all animals was not possible. In the poststimulated section of the experiment, all of the mice tested gave a positive indication of Pseudomonas aeruginosa. On the other hand, none of the mice in the prestimulated section tested positive for any pathogens.

The enhanced sensitivity of the poststimulated groups is of particular interest. It would appear that these mice were particularly susceptible to infection, more so even than their controls. This implies a cause of death due to an effect not on the erythrocytic cells, which were the elements stimulated to differentiate, but rather on the leukocytic cells, which are involved in the elimination of pathogenic organisms. Further, since anemia is not known to be a primary cause of death in the irradiated

mouse, it is doubtful that the observed change in sensitivity can be directly related to the levels of mature red cells in the irradiated animals. Moreover, Gurney[6] has observed on several occasions that transfused polycythemic mice demonstrate poorer survival following 400 or 500 R total body x ray than do nonpolycythemic, or normal, animals. This being the case, one must look elsewhere than the red cell level in order to find an explanation for the present observation.

It has been known for some time that radiation has a deleterious effect on the hematopoietic proliferative compartment. The work of Till and McCulloch[13,18] has demonstrated that it is possible to relate survival probability in mice to the number of hematopoietic colony-forming units which may be detected in the proliferative compartment after irradiation. Hence, this compartment, which contains the hematopoietic stem cells, becomes a likely candidate in which to find an explanation for the altered radiation sensitivities. In this regard, the findings given here agree with the observation of Kretchmar et al.,[10] who reported that preirradiation hypoxia exposure similar to that employed herein results in a 1.7-fold increase in hematopoietic colony-forming units. They are, on the other hand, at variance with the work of Bruce and McCulloch[5] who reported no such increase. The reason for this variance is not certain. Nonetheless, it does not rule out the possibility that by altering the demand for erythroid differentiation, one might also alter the rate of production of leukocytes. This question is particularly important in view of the work of McCulloch[12] who has demonstrated the existence of a common precursor cell for the erythroid, myeloid and thromboid lines, and the work of van Bekkum[20] who has related the lymphocyte to this same precursor cell. One might therefore expect that

a sustained increase in demand for red cell production could result in fewer precursor cells being made available for other types of hematopoiesis, while a decrease in demand might result in more precursor cells being made available for leukopoiesis. In the present experiments, the prestimulated mice are at a minimum level of erythropoiesis during and following irradiation exposure, while the poststimulated animals are at an elevated level. Thus our findings are in agreement with this interpretation. They do not, however, constitute a final proof of the interpretation as stated and further work directly at the precursor cell level is therefore warranted.

V. SUMMARY

Experiments have been performed for the purpose of evaluating the effect of chronic preirradiation erythropoietic stimulation and chronic postirradiation erythropoietic stimulation on survival in the mouse following x irradiation at exposures between 675 R and 775 R. It was found that the median survival time was shortened by postirradiation stimulation, but was markedly improved by preirradiation stimulation. In addition, a significant increase in survival in the prestimulated animals was noted over that demonstrated by unstimulated controls.

ADDENDUM

Subsequent to the completion of the experiments reported herein and the writing and editing of this report, an additional reference came to our attention which bears a definite relationship to the present work and is worthy of comment.

It has been observed by Tribukait and Forssberg* that following short term exposure to hypoxia (4 to 10 days at 6000 m) the radiation sensitivity is also affected in the CBA mouse by hypoxia exposure prior to irradiation. The experimental approach was somewhat different than that used here, but the results are generally in agreement. Whereas in the present paper the radiation exposure levels were varied, but the time of exposure after hypoxia was kept constant, in the work of Tribukait and Forssberg the radiation exposure was kept constant (756 R) but the time was varied. These authors report that compared to unstimulated controls, survival is reduced if irradiation occurs 1 hour after removal from hypoxia, but increased if it occurs between 24 and 96 hours after hypoxia exposure, the maximum survival increase being found with exposure at 72 hours and the minimum survival increase with exposure at 96 hours.

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13 ABSTRACT

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